



ELSEVIER

Aquatic Botany 82 (2005) 250–268

**Aquatic  
botany**

www.elsevier.com/locate/aquabot

## Effect of soil flooding on photosynthesis, carbohydrate partitioning and nutrient uptake in the invasive exotic *Lepidium latifolium*

Hongjun Chen<sup>a</sup>, Robert G. Qualls<sup>b,\*</sup>, Robert R. Blank<sup>c</sup>

<sup>a</sup> Wetland Biogeochemistry Institute, School of the Coast and Environment,  
Louisiana State University, Baton Rouge, LA 70803, USA

<sup>b</sup> Department of Natural Resources and Environmental Sciences, MS 370,  
University of Nevada, Reno, NV 89557, USA

<sup>c</sup> Exotic and Invasive Weed Research Unit, USDA-Agricultural Research Service,  
920 Valley Road, Reno, NV 89557, USA

Received 16 March 2004; received in revised form 24 February 2005; accepted 24 February 2005

### Abstract

*Lepidium latifolium* L. is an invasive exotic crucifer that has spread explosively in wetlands and riparian areas of the western United States. To understand the ecophysiological characteristics of *L. latifolium* that affect its ability to invade riparian areas and wetlands, we examined photosynthesis, chlorophyll concentration, carbohydrate partitioning and nutrient uptake in *L. latifolium* in response to soil flooding. Photosynthesis of flooded plants was about 60–70% of the rate of unflooded controls. Chlorophyll concentrations of flooded plants were about 60–70% of the unflooded plants during 15–50 days of flooding. Flooding resulted in an increase in leaf starch concentration, but root starch concentration was not significantly affected. However, concentrations of soluble sugar were significantly higher in both leaves and roots of flooded plants than unflooded controls. On day 50 after initial flooding, the concentrations of N, P, K and Zn in leaves of flooded plants were lower than in control plants. The concentrations of Mn and Fe in leaves of flooded plants were eight and two times those of control plants, respectively. In contrast, N, P, K and Zn concentrations of roots of flooded plants were slightly higher than in unflooded plants. The concentrations of Fe and Mn in roots of flooded plants were 15 and 150 times those of the control plants, respectively. The transport of P, K, and Zn to shoots decreased and that of Mn increased under flooding. The accumulation of N, K and Zn in roots decreased and that of Mn increased in response to flooding. The results suggested that the

\* Corresponding author. Tel.: +1 775 327 5014; fax: +1 775 784 4789.  
E-mail address: qualls@unr.edu (R.G. Qualls).

maintenance of relatively high photosynthesis and the accumulation of soluble sugar in roots of flooded plants are important adaptations for this species in flooded environments. Despite a reduction in photosynthesis and disruption in nutrient and photosynthate allocation in response to flooding, *L. latifolium* was able to survive 50 days of flooding stress. Overall, *L. latifolium* performed like a facultative hydrophyte species under flooding.

© 2005 Elsevier B.V. All rights reserved.

**Keywords:** Flood tolerance; Carbohydrate reserve; Photosynthesis; Nutrient transport; Nutrient accumulation; Wetland; Invasive species

---

## 1. Introduction

*Lepidium latifolium* L., perennial pepperweed or tall whitetop, is an invasive exotic crucifer that is widely distributed in the western United States (Young et al., 1995). This invasive species is classed as a noxious weed by the US Bureau of Land Management and by 10 states of the western United States. It is extremely competitive in many habitats, especially in riparian areas and wetlands. *L. latifolium* has been found to tolerate extensive flooding and increased salinity (Chen et al., 2002; Young et al., 1995). However, little is known about the ecophysiology of this species because of its relatively recent expansion.

Soil flooding reduces the photosynthetic rate of many plant species (Bradford, 1983; Pezeshki et al., 1996a; Gravatt and Kirby, 1998), particularly of flood-intolerant species. The decline in photosynthesis under flooding has been attributed to stomatal closure (Bradford, 1983), leaf chlorophyll decrease (Bradford, 1983), ethylene production (Pallas and Kays, 1982), reduced sink demand (Wample and Thornton, 1984), and disruption in photosynthate transport (Sij and Swanson, 1973). Under flooding, reduced photosynthesis is generally accompanied by the accumulation of non-structural carbohydrate in seedlings of flood-intolerant woody species (Vu and Yelenosky, 1991; Gravatt and Kirby, 1998). Starch accumulation has been reported in sunflower (*Helianthus annuus* L.) under hypoxic (oxygen deficient) conditions (Wample and Davies, 1983). Decreased rate of phloem transport to roots has been observed in flood-stressed crop species (Sij and Swanson, 1973), which has been viewed as one cause of starch accumulation (Topa and Cheeseman, 1992). However, the mechanisms involved in photosynthate translocation in flooded plants are poorly understood (Pezeshki, 1995, 2001).

Soil flooding causes oxygen deficiency (hypoxia), thus leading to reducing soil conditions (Ponnamperuma, 1972), and adversely affects nutrient uptake in plants (Pezeshki et al., 1999). The mineral nutrition of plants in response to flooding depends on plant species and soil type (Kozłowski, 1984; Pezeshki, 2001). In flood-intolerant species, the concentrations of N, P and K in foliages are often, but not always reduced by flooding (Kozłowski, 1984; Pezeshki, 1995). The effect of flooding on P uptake is complicated and is strongly dependent on soil type. In some alkaline soils that are relatively low in P, flooding may cause an increase in P availability, leading to a temporary increase in P concentration in plants, but prolonged flooding reduces P concentration and uptake in plants, due to root dysfunction, damage and death (Kozłowski, 1984). Ca and Mg uptake is altered less than that of N, P or K by flooding (Kozłowski, 1984). When flooded, however,

flood-tolerant species generally acquire more minerals than flood-intolerant species (Pezeshki et al., 1999). Uptake of Fe and Mn depends on plant species and their availability in soils, but generally significantly increases with flooding (Graven et al., 1965; Jones, 1972).

These changes in photosynthesis, carbohydrate partitioning and nutrient uptake result in the accumulation of mineral nutrients in roots and the inhibition of photosynthate translocation to roots. The disruption of inherent root–shoot communication caused by flooding reflects a functional disequilibrium between roots and shoots in flooded plants. Soil flooding has been proposed to control *L. latifolium* (Fredrickson et al., 1999). Therefore, a comprehensive knowledge of photosynthesis, carbohydrate partitioning and nutrient uptake under soil flooding is essential in understanding the invasion characteristics of *L. latifolium* in riparian areas and wetlands. Moreover, this knowledge might assist in finding new control strategies for this species. Our objective of this study was to show what adaptations this economically and ecologically important invasive species manifests to soil flooding. We hypothesized that: (1) net photosynthesis would be reduced under water-saturated soil conditions; (2) flooding would lead to higher carbohydrate concentrations in leaves but lower carbohydrate concentrations in roots of flooded plants; and (3) soil flooding would decrease the concentrations of N, P, K and Zn in leaves, but increase the concentrations of Fe and Mn in both leaves and roots. To evaluate these hypotheses, we (a) measured photosynthesis and chlorophyll concentration in leaves in response to soil flooding; (b) determined the concentrations of soluble sugar and starch in leaves, stems and roots in response to soil flooding; and (c) measured the concentrations of N, P, K, Fe, Mn and Zn in leaves and roots of flooded and unflooded plants.

## 2. Materials and methods

### 2.1. Plant material

*L. latifolium* is a perennial herbaceous plant in the mustard family that is native to southeastern Europe and western Asia (Munz and Keck, 1959). It forms dense colonies by adventitious shoots from deep-seated rhizomes. It grows from 0.5 to 1 m tall and has an extensive root system. Leaves are 10–30 cm long and 5–8 cm wide. The fruit is a silicle 2–2.5 mm long, with two seeds. It begins growth in late winter, first forming a rosette with stems developing later. By late spring, shoots begin to flower and then produce seed. During flowering and seed maturation, many leaves begin to senesce. In the United States, where it is an exotic, *L. latifolium* is considered an aggressive colonizer of wetland and riparian areas (Young et al., 1995).

Seeds of *L. latifolium* were collected from wild plants at the University of Nevada Farm in Reno, NV, USA. They were germinated in washed sand in an aluminum tray in a glasshouse, and then watered using half-strength Hoagland's solution (Lindsay, 1991). Seedlings were transferred to 10 cm × 10 cm × 9 cm pots containing natural riparian loam soil of the Truckee series, a fine-loamy, mixed (calcareous), mesic Fluvaquent Haplaquoll, from the University of Nevada Farm. Afterwards, small plants, with 3–5 mature leaves and roots of uniform length, were selected and randomly transplanted into 20

plastic barrels (56 cm diameter and 72 cm height) containing homogenized soil of the same series.

Each barrel was placed outside, with six plants spaced at equal distances from one another in each barrel. Before initiation of the soil flooding treatment, plants were allowed to grow, a soil water potential of  $-20$  kPa was maintained for 7-day pretreatment and soil water potential was monitored using a tensiometer. Each barrel was fitted with two tensiometers, one at 15 cm from the surface and another at 15 cm from the bottom. After the 7-day period of pretreatment, 15 of the 20 barrels were randomly assigned to the soil flooding treatment. Soil in the 15 barrels was flooded to about 1 cm above the soil surface. The other five barrels were used as unflooded controls. We used more barrels and plants for the flooded treatment than that for the control because we thought that there might be considerable mortality in the flooded plants and that the stress of flooding might cause more variability in response among plants. Soil in the five unflooded barrels was kept at a soil water potential of  $-20$  kPa. Soil flooding treatment included five durations of 3, 7, 15, 30 and 50 days, with 18 replicates (plants) in three flooded barrels and six plants in one unflooded control barrel for each treatment duration. On days 3, 7, 15, 30 and 50 after initial flooding, all plants from randomly selected barrels were harvested, and tissues were collected and dried at  $70^{\circ}\text{C}$  for 72 h for the measurement of biomass, carbohydrates and nutrients. Analyses of all samples were performed in duplicate except where indicated.

## 2.2. *Photosynthesis*

On days 3, 7, 15, 30, 42 and 50 after initial flooding, photosynthesis of single, attached, mature leaves (one leaf on each of three different plants in one barrel per control and one leaf on each of nine different plants in three barrels per treatment for each treatment duration) was measured between 12:00 and 13:00 under natural solar irradiance ( $1800\text{--}2000\ \mu\text{mol m}^{-2}\text{ s}^{-1}$ ), using a LICOR 6200 Portable Photosynthesis System. Photosynthesis was calculated per unit leaf area (single surface). The area of the tested leaf was traced on a piece of paper and then the area of the paper was determined with a LICOR LI-3000A leaf area meter.

## 2.3. *Chlorophyll*

On each sampling date, leaves (one leaf on each of three different plants in one barrel per control and one leaf on each of nine different plants in three barrels per treatment for each duration) were collected, immediately frozen in liquid nitrogen and stored in a freezer at  $-79^{\circ}\text{C}$  until analysis. Frozen leaf samples were ground and extracted with 85% (v/v) acetone and chlorophyll concentration was determined by the method of [Arnon \(1949\)](#).

## 2.4. *Carbohydrates*

On each sampling date, leaf, stem and root samples were collected in a design similar to that described for chlorophyll, and subsampled for carbohydrate determination. Approximately 0.05 g of the sample was extracted with boiling 80% ethanol and incubated at room temperature overnight ([Gravatt and Kirby, 1998](#)). After centrifugation,

the extracted residue was washed with 80% ethanol three times. The combined supernatants were collected, heated until free of ethanol and then brought to 10 ml with deionized water. These solutions were centrifuged at  $3600 \times g$  for 30 min and the supernatant was used for assay of soluble sugars by the phenol–sulfuric acid method (Dubois et al., 1956). The ethanol-insoluble fraction above was oven-dried at  $70^\circ\text{C}$  for 48 h, and then extracted with 100 mM acetate buffer, pH 4.8 (Carter et al., 1973). The homogenate was incubated with 100 mg of amyloglucosidase at room temperature overnight. Samples were subsequently centrifuged. Starch was estimated as glucose liberated and analyzed by the phenol–sulfuric acid method (Dubois et al., 1956). For each set of samples, a standard curve was expressed as glucose equivalents ( $\text{mg glucose g}^{-1}$  dry weight).

### 2.5. Nutrient analysis

On each sampling date, leaf, stem and root samples were collected, subsampled and dried at  $60^\circ\text{C}$  for elemental analysis. The concentrations ( $\mu\text{mol g}^{-1}$ ) of N, P, K, Fe, Mn and Zn were determined for roots and leaves on ground tissue. Nitrogen was determined with a Perkin-Elmer PE 2400 CHN analyzer. Samples for the determination of P, K, Fe, Mn and Zn were ashed at  $500^\circ\text{C}$  for 4 h, dissolved in 1 M HCl, and analyzed for P by an ammonium molybdate–ascorbic acid procedure (Murphy and Riley, 1962), for K by atomic emission spectrometry and Zn, Fe and Mn by atomic absorption spectrometry.

Nutrient transport to the shoot is defined here as the total amount of the nutrient ( $\mu\text{mol}$ ) transported to the shoot per root dry weight (g) per day. Nutrient accumulation in the root is defined as the total amount of nutrient ( $\mu\text{mol}$ ) taken up into the root per root dry weight (g) per day. The equation used was taken from Hunt (1982):

$$\text{transport or accumulation} = \frac{(M_2 - M_1)(\ln W_2 - \ln W_1)}{(T_2 - T_1)(W_2 - W_1)}$$

where  $M$  is the total amount of the nutrient in either the shoot (transport) or root (accumulation),  $T$  is time, and  $W$  is the root weight. Subscripts refer to the parameter value at that particular harvest date. Transport and accumulation are expressed in units of  $\mu\text{mol g}^{-1}$  dry weight  $\text{day}^{-1}$ . Data were calculated using the “combined approach” (Poorter, 1989) in a similar manner to that used for relative growth rate.

### 2.6. Statistical analysis

The experiment consisted of two factors, one categorical (flooding treatment) and one metric (treatment duration). Data on photosynthesis, chlorophyll concentration, carbohydrate concentration, nutrient concentration, and nutrient transport or accumulation rate were analyzed with an analysis of covariance (ANCOVA), using the general linear model (GLM) procedure of the Statistical Analysis System (SAS Institute Inc., Cary, NC). Variation was partitioned into two main effects and their interactions: the flooding treatment and duration.

### 3. Results

#### 3.1. Photosynthesis

Photosynthesis declined in response to soil flooding treatment (Fig. 1A). After 3 days of flooding, the photosynthesis of flooded plants was 72% of that of the unflooded controls. On day 42, photosynthesis in flooded plants was about 56% of the rate in control plants. Later, unflooded plants began to exhibit senescent symptoms, due to the end of the growing season and the beginning of reproduction. Unflooded plants began to flower and then produced many seeds while flooded plants did not. On day 50, photosynthesis was low with no significant difference between flooded and unflooded plants. Analysis of covariance showed that flooding treatment significantly influenced photosynthesis ( $F = 8.84$ ,  $P = 0.004$ ), but there was no significant effect of the treatment duration ( $F = 0.07$ ,  $P = 0.79$ ), and the effect of their interaction was not significant ( $F = 0.71$ ,  $P = 0.40$ ) (Table 1).

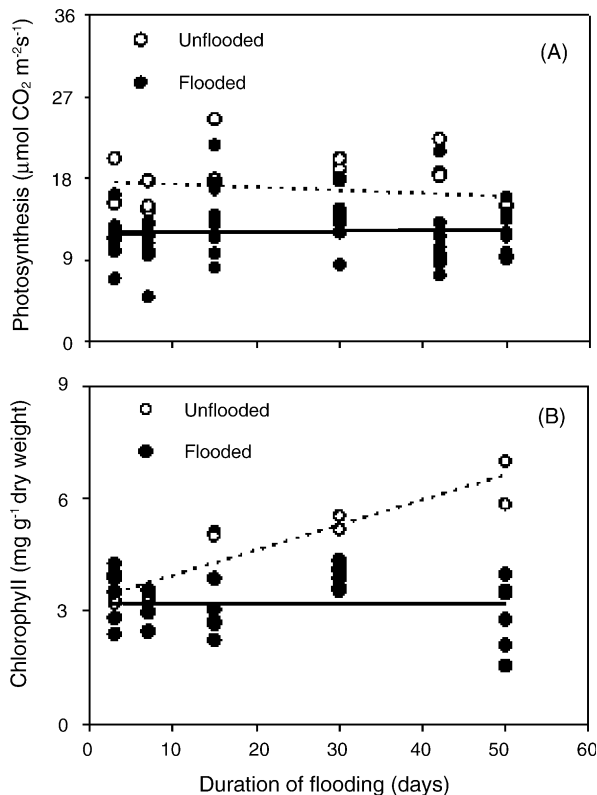


Fig. 1. Photosynthesis and chlorophyll concentration (mean  $\pm$  S.E.) in leaves of *Lepidium latifolium* in response to soil flooding. Dashed lines show regression lines for the unflooded treatment, while solid lines represent regression lines for the flooded treatment.

Table 1  
Analysis of covariance of photosynthesis, chlorophyll, soluble sugar and starch

Variables	Sources	<i>F</i> -values	<i>P</i> -values
Photosynthesis	Flooding (F)	8.84	0.004
	Duration (D)	0.07	0.788
	F × D	0.71	0.401
Chlorophyll	Flooding	3.20	0.082
	Duration	21.4	0.001
	F × D	22.5	0.001
Soluble sugar in leaves	Flooding	4.62	0.047
	Duration	0.01	0.919
	F × D	0.65	0.431
Starch in leaves	Flooding	0.01	0.930
	Duration	2.45	0.137
	F × D	3.07	0.099
Soluble sugar in roots	Flooding	5.33	0.035
	Duration	3.88	0.067
	F × D	0.37	0.552
Starch in roots	Flooding	1.67	0.215
	Duration	21.5	0.001
	F × D	2.21	0.156

The degree of freedom for main effects (flooding and duration of flooding) and their interactions is one. Note that duration of flooding was treated as a continuous variable, rather than a categorical variable.

### 3.2. Chlorophyll

Soil saturation caused a decline in chlorophyll concentration (Fig. 1B). In the first 7 days of flooding, there were no large differences in chlorophyll between unflooded and flooded plants. However, during 15–50 days of flooding, chlorophyll concentrations of flooded plants were about 60–70% of those of the controls. ANCOVA showed that there was a significant effect of the treatment duration ( $F = 21.41$ ,  $P < 0.001$ ) and a significant interaction of flooding treatment and treatment duration ( $F = 22.46$ ,  $P < 0.001$ ) on chlorophyll concentration (Table 1).

### 3.3. Carbohydrate partitioning

Mean soluble sugar concentration was significantly greater in leaves, stems and roots of flooded plants than in the unflooded controls (Tables 1 and 2). On days 3, 7, 15, 30 and 50, leaf soluble sugar concentration in flooded treatments was 130, 188, 132, 130 and 142%, respectively, and root soluble sugar was 128, 320, 173, 161 and 120%, respectively, of concentrations in control plants (Table 2). ANCOVA showed that there was a significant effect of flooding treatment on soluble sugar in leaves and in roots, but there was no significant effect of the treatment duration or the interaction was found (Table 1). There was a significant difference in soluble sugar concentration in stems between flooded and control plants on day 50, the only time when there was a substantial mass of stem tissue (Table 2).

Table 2

Soluble sugar and starch concentrations (mean  $\pm$  S.E.,  $n = 3$ ) in *Lepidium latifolium* in response to soil flooding

Organ	Duration (days)	Soluble sugar (mg glucose g <sup>-1</sup> dry weight)		Starch (mg glucose g <sup>-1</sup> dry weight)	
		Unflooded	Flooded	Unflooded	Flooded
Root	3	89.9 $\pm$ 8.0	116 $\pm$ 7	760 $\pm$ 31	724 $\pm$ 24
	7	69.1 $\pm$ 7.1	130 $\pm$ 5	646 $\pm$ 19	775 $\pm$ 30
	15	66.0 $\pm$ 5.5	87.3 $\pm$ 7.8	797 $\pm$ 22	799 $\pm$ 28
	30	74.0 $\pm$ 7.3	95.8 $\pm$ 7.3	862 $\pm$ 25	868 $\pm$ 28
	50	68.9 $\pm$ 6.1	97.9 $\pm$ 4.7	951 $\pm$ 24	831 $\pm$ 26
Leaf	3	84.5 $\pm$ 7.5	108 $\pm$ 14	735 $\pm$ 27	715 $\pm$ 21
	7	52.1 $\pm$ 4.9	167 $\pm$ 12	623 $\pm$ 17	749 $\pm$ 54
	15	71.1 $\pm$ 6.7	123 $\pm$ 2	718 $\pm$ 25	858 $\pm$ 27
	30	56.6 $\pm$ 3.9	90.9 $\pm$ 3.1	704 $\pm$ 15	925 $\pm$ 20
	50	106 $\pm$ 8	128 $\pm$ 7	683 $\pm$ 23	834 $\pm$ 31
Stem	50	71.5 $\pm$ 6.6	83 $\pm$ 3.2*	724 $\pm$ 26	678 $\pm$ 10*

Difference in stem carbohydrate between flooded and unflooded plants is represented by Student's *t*-test.\*  $P < 0.05$ .

Flooding did not significantly increase the concentration of starch in leaves and roots (Tables 1 and 2). During 7–50 days of flooding, leaf starch concentration in flooded plants was increased by 20–30%, compared to unflooded plants, but ANCOVA showed that this difference is not statistically significant (Table 1). In roots, the treatment duration significantly influenced starch concentration in flooded and control plants. It appeared that starch concentration increased with the time in unflooded and flooded plants. In stems of flooded plants starch concentration was significantly lower than in unflooded controls (Table 2).

### 3.4. Nutrient concentration in leaves

How flooding treatment influenced nutrient concentrations in the leaves of *L. latifolium* was element-specific. ANCOVA showed that there was a significant effect of flooding treatment on the concentrations of N, P and Fe in leaves, while the flooding treatment effect on K, Mn and Zn was not significant (Table 3). In some cases (such as K) the effects of flooding treatment were different over time as reflected in a significant interaction of flooding treatment and treatment duration (Table 3). On day 3 after flooding, the concentrations of all nutrients in leaves of flooded plants were not markedly different from unflooded plants (Fig. 2). However, on days 15 and 30 the concentrations of N, P, K, and Zn in leaves of flooded plants were significantly and consistently lower than unflooded controls. On day 50, there was no significant difference in N between flooded and unflooded plants, whereas the concentrations of P, K and Zn were 40–50% lower in flooded plants than in unflooded controls. Leaves of flooded plants had higher Mn concentration than unflooded controls 3 days after flooding. On day 50, Mn concentration was about eight times that of the control plants. The Fe concentration in leaves was consistently greater in leaves of the flooded treatment (Fig. 2) and ANCOVA indicated a significant effect of flooding treatment (Table 3).

Table 3  
Analysis of covariance of nutrient concentration, transport and accumulation

Sources	N		P		K		Fe		Mn		Zn	
	F	P	F	P	F	P	F	P	F	P	F	P
Concentration in leaves												
Flooding (F)	7.76	0.013	34.1	0.001	0.21	0.645	7.50	0.015	1.83	0.195	0.01	0.909
Duration (D)	50.8	0.001	21.1	0.001	0.11	0.749	24.9	0.001	24.9	0.001	1.55	0.230
F × D	0.02	0.896	1.96	0.181	17.3	0.001	2.30	0.149	23.9	0.002	6.88	0.018
Concentration in roots												
Flooding	18.1	0.001	5.48	0.033	2.93	0.106	2.73	0.118	2.59	0.127	3.16	0.094
Duration	134	0.001	1.80	0.198	33.9	0.001	0.98	0.338	8.20	0.011	22.9	0.001
F × D	10.68	0.005	6.06	0.026	0.10	0.755	13.3	0.002	12.2	0.003	0.53	0.477
Transport to leaves												
Flooding	2.66	0.122	11.0	0.004	6.43	0.022	2.71	0.119	87.3	0.001	8.04	0.012
Duration	11.3	0.004	18.1	0.001	9.81	0.006	1.18	0.003	70.8	0.001	15.6	0.001
F × D	1.57	0.228	6.52	0.021	2.89	0.108	3.11	0.097	47.5	0.001	1.37	0.259
Accumulation in roots												
Flooding	5.81	0.028	3.27	0.089	11.3	0.004	10.3	0.005	0.02	0.899	3.44	0.082
Duration	17.2	0.001	14.6	0.002	17.8	0.001	5.63	0.031	0.24	0.628	5.39	0.034
F × D	2.96	0.105	1.43	0.249	6.01	0.026	14.2	0.002	8.16	0.011	1.19	0.292

The degree of freedom for main effects and their interactions is one. Note that the duration of flooding was treated as a continuous variable rather than a categorical variable.

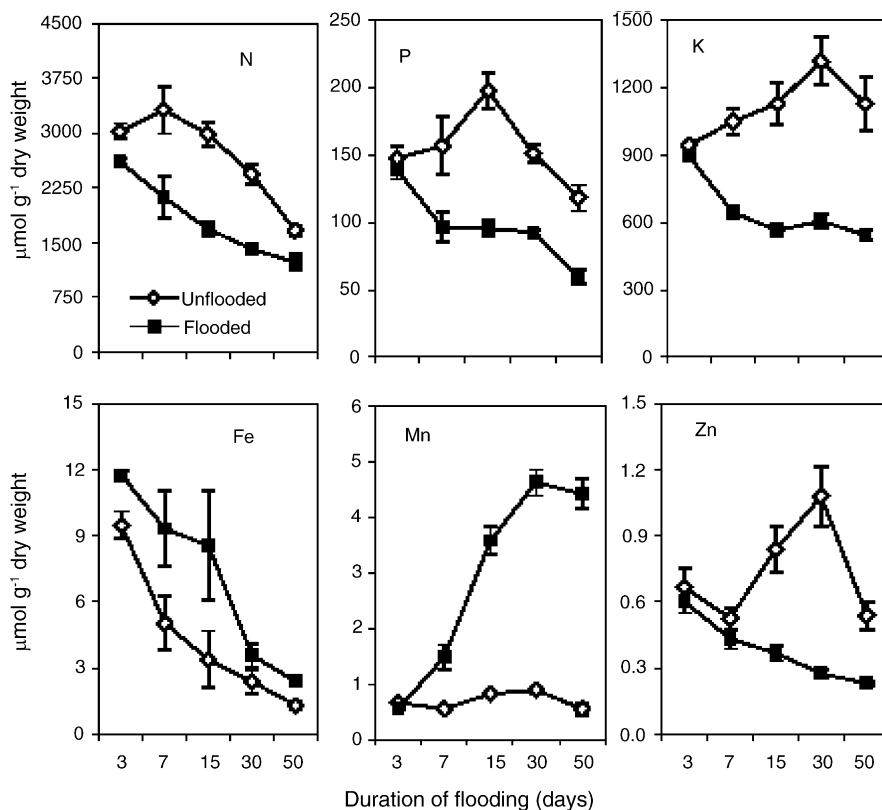


Fig. 2. Effect of soil flooding on leaf nutrient concentration ( $n = 3$ ) in *Lepidium latifolium*. Bars represent  $\pm$ S.E. and are smaller than the symbol if not shown.

The treatment duration significantly influenced the concentration of N, P, Fe, and Mn in leaves, but had little effect on that of K and Zn (Table 3). The concentration of N, P, Fe, and Zn in flooded plants decreased with treatment duration throughout the 50 days of flooding (Fig. 2). The concentration of N, P, Fe and Zn was reduced by 53, 57, 80 and 75% between days 3 and 50, respectively. In contrast, Mn concentration in flooded plants dramatically increased from 0.55 to 4.42  $\mu\text{mol g}^{-1}$  dry weight between days 3 and 50. Also, there was a significant interaction of flooding treatment and the treatment duration on the concentration of K, Mn and Zn in leaves (Table 3).

### 3.5. Nutrient concentration in roots

On day 3 after flooding, the concentrations of all nutrients in roots of flooded plants were not markedly different from unflooded plants except for N and Zn (Fig. 3). Root N concentrations decreased throughout the experiment in both flooded and unflooded treatments. Nitrogen concentrations in flooded plants were slightly lower than in unflooded plants except for day 50. The concentrations of K and Zn were significantly lower in

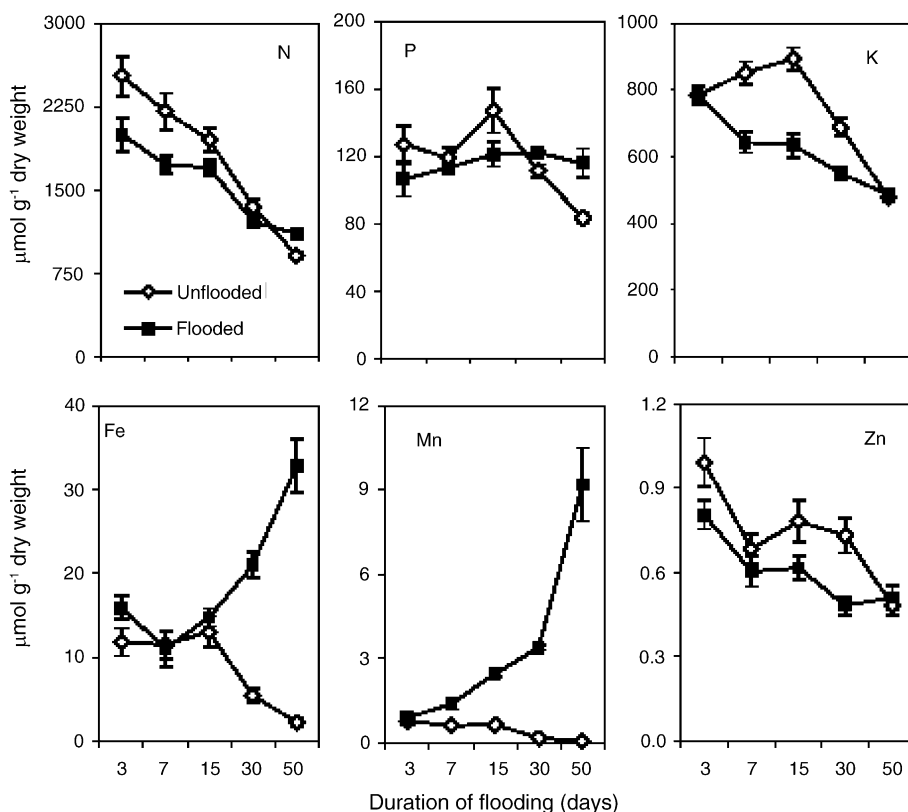


Fig. 3. Effect of soil flooding on root nutrient concentration ( $n = 3$ ) in *Lepidium latifolium*. Bars represent  $\pm$ S.E. and are smaller than the symbol if not shown.

flooded plants than in control plants 3 days after flooding, but there was no significant difference in K or Zn between flooded and unflooded plants on day 50. In contrast, the concentrations of Fe and Mn in flooded roots were significantly higher than in unflooded controls 15 days after flooding, and increased with the duration of flooding. On day 50, the concentrations of Fe and Mn in roots were about 15 and 150 times those of controls, respectively. ANCOVA indicated that there was a significant effect of flooding treatment on the concentrations of N and P, and the treatment duration on that of N, K, Mn and Zn. A significant interaction effect was also found on the concentration of N, P, Fe and Mn (Table 3).

### 3.6. Nutrient transport to shoots and accumulation in roots

Soil flooding significantly affected the rate of transport of all nutrients to shoots except for N and Fe, while the effect of the treatment duration was significant on the transport rate of all nutrients (Table 3). Also, a significant interaction between flooding treatment and the treatment duration was found only on the transport rate of P and Mn. The rate of transport

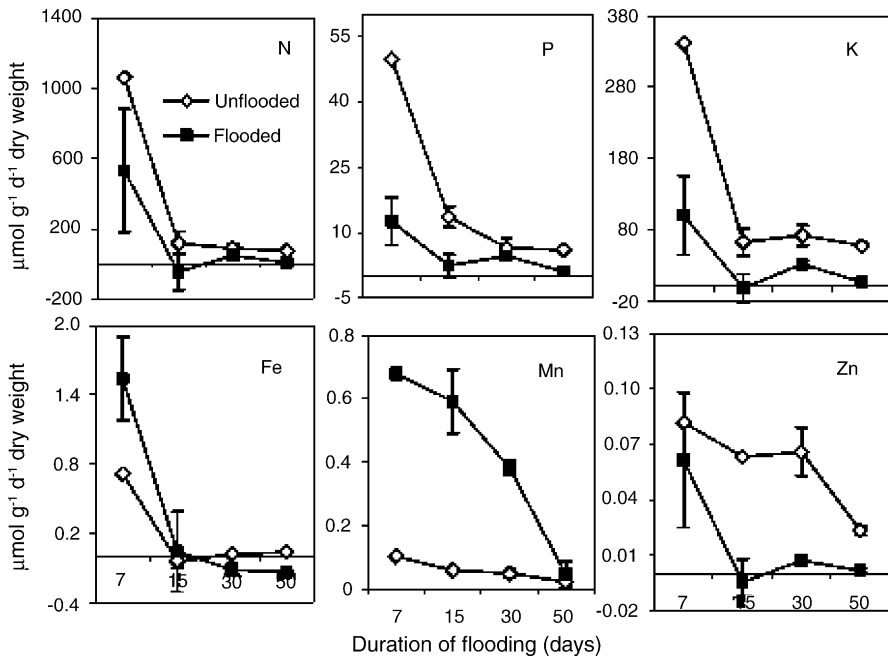


Fig. 4. Effect of soil flooding on nutrient transport ( $n = 3$ ) to leaves in *Lepidium latifolium*. Bars represent  $\pm$ S.E. and are smaller than the symbol if not shown.

of P, K and Zn to shoots was lower in flooded plants than in unflooded controls throughout 50 days of flooding (Fig. 4). On days 30 and 50, the rate of transport of Zn to shoots in flooded plants was 11 and 7% that of unflooded plants, respectively. In contrast, the rate of transport of Mn to shoots was much higher in flooded plants than in the unflooded controls throughout 50 days of flooding. On days 7, 15, 30 and 50, the rate of transport of Mn to shoots in flooded plants was 7, 10, 8 and 2 times that of unflooded controls, respectively.

Soil flooding significantly influenced the rates of accumulation of N, K and Fe in roots, but the effect of flooding treatment on the rate of accumulation of P, Mn and Zn was not significant (Table 3). The treatment duration significantly affected the accumulation rate of N, P, K and Zn in roots (Table 3). The temporal pattern of the accumulation rate of N, P, K and Zn in roots of flooded plants was similar throughout the 50 days of flooding (Fig. 5). On day 7, the rates of accumulation of those nutrients in flooded plants were highest, close to the unflooded plants, and the lowest on day 15 when they were only 2–10% of unflooded controls. Subsequently, their rates slowly increased on day 30, and slightly decreased on day 50. The interaction between the treatment duration and flooding treatment on the rate of accumulation of Fe was significant (Table 3) because the rates of Fe accumulation were lower on days 7 and 15, but then higher on days 30 and 50 in flooded plants (Fig. 5). In contrast, the rate of Mn accumulation in roots was significantly and consistently higher in flooded plants than in unflooded throughout the 50-day flooding (Fig. 5) as reflected in a significant interaction of flooding treatment and treatment duration (Table 3).

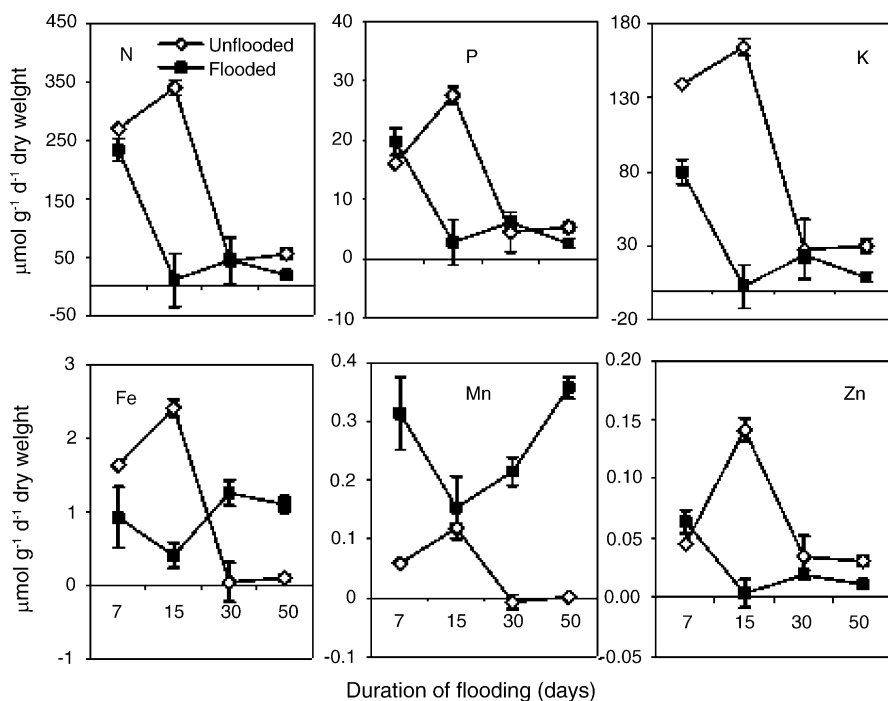


Fig. 5. Effect of soil flooding on nutrient accumulation ( $n = 3$ ) in roots in *Lepidium latifolium*. Bars represent  $\pm$ S.E. and are smaller than the symbol if not shown.

## 4. Discussion

### 4.1. Photosynthesis and carbohydrate concentrations

Photosynthesis of flooded *L. latifolium* was 56–72% of that in unflooded plants throughout the 50 days of flooding (Fig. 1A). During the first 7 days of flooding, photosynthesis was reduced in flooded plants while chlorophyll concentrations were similar to non-flooded plants (Fig. 1). This suggests that the initial effect of flooding on photosynthesis may not have been caused by leaf chlorophyll loss but by partial stomatal closure. It has been reported that many wetland species initially close stomata in response to soil flooding (Kozłowski, 1984). On days 15 and 30, the reduction in photosynthesis was similar to that in chlorophyll, suggesting a time lag of about two weeks in the effect on chlorophyll turnover. Finally, on day 50, we also observed a decrease in photosynthesis in the unflooded plants that we interpret as related to the beginnings of leaf senescence as those unflooded plants flowered and produced seed, although chlorophyll concentrations remained relatively high. Lower photosynthesis was also accompanied by lower relative growth rate and biomass, as described in a separate paper (Chen et al., 2002).

The extent of the effect of flooding on photosynthesis in this study was similar to that of seedlings of *Nyssa aquatica* L. (Gravatt and Kirby, 1998), cattail (*Typha domingensis*) and sawgrass (*Cladium jamaicense*); all flood-tolerant species (Pezeshiki et al., 1996a). In

contrast, flood-intolerant species have displayed an appreciable drop in photosynthesis falling to only 5–25% of unflooded controls (Gravatt and Kirby, 1998; Pezeshki et al., 1996b). Stomatal closure appears to be mainly responsible for the decrease in photosynthesis during short-term submergence, but is not the only limiting factor (Bradford, 1983). One factor restricting photosynthetic activity may include increased ethylene production under flooding stress (Pallas and Kays, 1982; Taylor and Gunderson, 1988). In our previous study, we found that ethylene production significantly increased in *L. latifolium* during the flooding period (Chen et al., 2002). This increase in ethylene production may have caused chlorosis and premature senescence of leaf tissues, thus leading to a decrease in photosynthetic rate. In addition, a decrease in root growth of *L. latifolium* under flooding stress (Chen et al., 2002) reduces root sink demand for photosynthates, which has been viewed as one important factor restricting photosynthesis (Wample and Thornton, 1984). Other factors may involve disruption in photosynthate transport (Sij and Swanson, 1973).

Under flooding, the reduction in photosynthesis was accompanied by increased concentrations of soluble sugar and starch in leaves (Gravatt and Kirby, 1998). In roots of flooded plants we also found greater concentration of soluble sugar but not starch (Table 2). The effect of flooding on starch accumulation in *L. latifolium* resembled that found in seedlings of *Fraxinus pennsylvanica* Marshall, a moderately flood-tolerant woody species (Gravatt and Kirby, 1998). Similar results have been reported in sunflower plants (Wample and Davies, 1983). Therefore, photosynthate delivery from leaves (the source) to roots (the sink) may be inhibited under flooding stress, resulting in carbohydrate accumulation in leaves of flooded plants. The inhibition of the rate of carbohydrate translocation may be much greater than the decrease in net photosynthesis. It is well known that the decrease in carbohydrate demand in roots under anoxia has an adverse influence on phloem import into roots (Geiger and Savonick, 1975; Saglio, 1985). In a previous study (Chen et al., 2002), the reduced root growth of *L. latifolium* under flooding led to a change in the root/shoot ratio from 1.15 in unflooded controls to 0.60 in flooded plants during 50 days of flooding. Reduction in root growth and shoot elongation as a flowering response may cause a decrease in root carbohydrate demand, which may be one cause of reduced photosynthate transport to flooded roots.

The accumulation of soluble sugar in roots of *L. latifolium* suggests that the availability of soluble sugar is not limiting to metabolic activity in roots. The primary use of carbohydrate reserves is to maintain respiration and growth in the absence of photosynthesis (Hook and Brown, 1973). The supply of soluble sugar allows roots to respire anaerobically under flooding conditions. The observation that an exogenous supply of glucose enhances survival of crop plants under flooding stress (Webb and Armstrong, 1983) has confirmed this importance. In the roots of *L. latifolium* we observed a rapid development of aerenchyma in response to anoxic conditions, and an increase in ethylene concentrations (Chen et al., 2002). Increases in the activity of alcohol dehydrogenase and the concentration of ethanol suggested that fermentative metabolism was occurring in roots (Chen and Qualls, 2003). However, the activity of both alcohol dehydrogenase and cytochrome oxidase suggested the concurrent activity of both fermentation and aerobic metabolism in different portions of the roots (Chen and Qualls, 2003). Because of the inefficient use of glucose in fermentation, it might seem that demand for soluble sugar

would increase and that carbohydrate reserves might be depleted during fermentative metabolism in roots, but the pattern of increase in carbohydrates under flooding in some species is common (Gravatt and Kirby, 1998). The increase in soluble sugar in roots in *L. latifolium* compared to unflooded controls was rapid and was greatest at 7 days after flooding, the same period at which we observed maximum ethylene production and the greatest rate of increase on root porosity (Chen et al., 2002). Consequently, the increase in soluble sugar might simply have been a product of the ethylene-induced enzymatic breakdown in root cellulose to produce aerenchyma.

The pattern of starch allocation in roots of flooded plants depends on the type of species. Under flooding stress, flood-tolerant *N. aquatica* exhibits much higher concentrations of starch in roots of flooded seedlings than unflooded plants, while flood-intolerant *Quercus alba* has much lower concentrations of starch in roots of flooded plants than unflooded plants (Gravatt and Kirby, 1998). Root carbohydrates are also an important source of materials for new root and leaf growth in the spring and after plants return to aerobic conditions (Crawford, 1978). It is possible that the failure of *L. latifolium* to deplete starch reserves in roots might be an adaptation that allows more rapid recovery on re-exposure to air after flooding as was suggested by Crawford (1978) for other species.

#### 4.2. Nutrient concentrations and allocation

In this study, the concentrations of N, P, K and Zn in leaves were lower in flooded plants than in unflooded plants. Concentrations of Fe and Mn in both leaves and roots were greater in response to soil flooding (Figs. 2 and 3). The pattern of nutrient response in *L. latifolium* to flooding was similar to that in flood-intolerant species such as seedlings of *Triticum aestivum* L. (Trought and Drew, 1980) and *Zea mays* L. (Lizaso et al., 2001). Leaf N concentration in flooded plants decreased significantly (Fig. 2) as has also been shown for *Glycine max* L. (Bacanamwo and Purcell, 1999). One study has shown that the delivery rate of nitrate from roots to shoots in flooded tomato (*Lycopersicum esculentum* L.) for 24 h was only 7% of that in well-drained plants (Else et al., 1995). Also, flood-induced microbial denitrification in soil decreases the supply of nitrate for plants (Gambrell et al., 1991). In the present study, leaf N concentration under flooding was reduced, but remained in the range of leaf N concentrations for optimal growth of crops, 1.4–3.6 mmol g<sup>-1</sup> dry weight (Marschner, 1995).

Flooded plants exhibited lower P concentration in leaves than unflooded controls (Fig. 2). The lower rate of P transport to shoots in flooded *L. latifolium* during the 3- and 7-day period of flooding treatment corresponded with the largest differences in P concentration in leaves (Fig. 4), suggesting that this lower rate of P transport in the early stages of flooding led to differences in P content. However, low rates of P transport to both flooded and unflooded leaves in the latter stages of the experiment led to declining concentrations over time in both treatments. It should be noted that the definition of “transport” and “accumulation” (see Section 2) is on per unit dry weight basis, so a “dilution” of nutrient concentration as the plant grows is often reflected in a decrease over time in nutrient transport. In seedlings of *Pinus serotina* Michaux, <sup>32</sup>P transport to shoots has been shown to be significantly lower under hypoxic conditions (Topa and Cheeseman, 1993). Also, enhanced Fe concentration in roots of flooded plants (Table 3) may interfere

with P uptake and immobilize P in roots, thus preventing further transport of P to shoots (McKevlin et al., 1987).

Prolonged flooding resulted in decreased concentration of K in leaves of flooded plants, which is supported by lower transport of K to shoots under flooding (Figs. 2 and 4). Extensive flooding caused the accumulation of Fe and Mn in *L. latifolium*, especially in roots (Fig. 4), an effect also shown to occur in flood-sensitive species (McKevlin et al., 1987; Pezeshki et al., 1999). Root oxygen deficiency decreases the selectivity of  $K^+/Na^+$  uptake by roots in favor of  $Na^+$  and retards the transport of  $K^+$  to shoots (Thomson et al., 1989). This change in the selectivity of ion uptake may be one cause of Na accumulation in flooded *L. latifolium* (data not shown). In this study, Zn concentration in leaves of flooded plants is very low, ranging from 0.2 to 0.4  $\mu\text{mol g}^{-1}$  dry weight (Fig. 2). This results from a significant decrease in the transport of Zn to shoots under flooding (Fig. 4). Compared with 1.0  $\mu\text{mol g}^{-1}$  dry weight, the average of 12 dicotyledonous herbs (Allen, 1989), Zn in flooded plants may be deficient.

In flooded soils, soluble  $Fe^{2+}$  and  $Mn^{2+}$  can lead to excessive uptake during prolonged periods of flooding (Ponnamperuma, 1972). If we compare the pattern of transport rate of Fe to shoots to that of accumulation rate in roots (Figs. 4 and 5), it appears that much of the Fe taken up by flooded plants was transported to the shoots initially, but later, after 30-day flooding, it was simply accumulated in the roots. The toxicity threshold of  $Fe^{2+}$  for wetland rice (*Oryza sativa* L.) is reportedly 8.9  $\mu\text{mol g}^{-1}$  leaf dry weight but is strongly dependent on species and other factors (Yamauchi, 1989). During the early period of growth in our experiment, the concentration of  $Fe^{2+}$  in leaves of flooded plants was about 10.5  $\mu\text{mol g}^{-1}$  dry weight (Fig. 2). We, therefore, suspect that the concentration of  $Fe^{2+}$  in flooded plants might have reached toxic levels in *L. latifolium*. In waterlogged *Epilobium hirsutum* L., enhanced  $Fe^{2+}$  uptake leads to the formation of reactive superoxide radicals and hydroxyl radicals by an iron-catalyzed reaction in roots, thus causing reduced protein synthesis, increased lipid peroxidation and gross cellular damage in roots (Hendry and Brocklebank, 1985). In our previous study, we also observed increased lipid peroxidation in roots of *L. latifolium* growing under anoxia (Chen and Qualls, 2003). Also, leaf Mn concentration in flooded plants was also high, compared with the critical toxic concentration for maize, 3.6  $\mu\text{mol g}^{-1}$  leaf dry weight (Edwards and Asher, 1982). Whether this level of Mn in flooded plants was toxic to *L. latifolium* is unknown and needs further study.

The pattern of nutrient transport to shoots of flooded *L. latifolium* also suggested that mineral nutrient ions such as Zn were not efficiently transported from roots (the source) to leaves (the sink) (Fig. 4). These ions that must be transported across the plasmalemma of root epidermal and cortical cells by an energy-driven process, are also inhibited by tissue anoxia (Saglio, 1985; Drew, 1988). The translocation of these nutrient ions is critical to understanding nutrient uptake in plants under flooding. However, the mechanism of nutrient ion translocation remains to be further evaluated (Pezeshki, 2001).

In short, soil flooding decreased the concentrations of N, P, K and Zn in leaves, but increased the concentrations of Fe and Mn in leaves and roots of *L. latifolium*. Under an extensive flooding stress, however, this species was capable to maintain about 60–70% of the photosynthesis of unflooded plants, had higher concentrations of soluble sugar in roots, and did not deplete starch reserves compared to unflooded plants. Despite reductions in growth, biomass (Chen et al., 2002) and photosynthesis, and disruptions in nutrient uptake

and photosynthate allocation, *L. latifolium* was able to survive 50 days of flooding and performed like a facultative hydrophyte species under soil flooding.

## Acknowledgements

We thank Akiko Takiyama for assistance in collecting samples, Vickie Smith-Longozo for tissue N analysis, Dr. Mark Walker for providing access to his laboratory, and Dr. Grant Cramer for critical comments on nutrient uptake. Funding for this project was provided by a grant from the US Department of Agriculture, National Research Initiative Competitive Grants Weed Science Program. Funding was also provided in part by the Nevada Agricultural Experiment Station.

## References

- Allen, S.E., 1989. Chemical Analysis of Ecological Materials. Blackwell, pp. 329–330.
- Arnon, D.I., 1949. Copper enzymes in isolated chloroplasts: Polyphenoloxidase in *Beta vulgaris*. Plant Physiol. 24, 1–15.
- Bacanamwo, M., Purcell, L.C., 1999. Soybean dry matter and N accumulation responses to flooding stress: N sources and hypoxia. J. Exp. Bot. 50, 689–696.
- Bradford, K.J., 1983. Effect of soil flooding on leaf gas exchange of plants. Plant Physiol. 73, 475–479.
- Carter, J.L., Garrard, L.A., West, S.H., 1973. Effect of gibberellic acid on starch degrading enzymes in leaves of *Digitaria decumbens*. Phytochemistry 12, 251–254.
- Chen, H., Qualls, R.G., Miller, G.C., 2002. Adaptive responses of *Lepidium latifolium* to soil flooding: biomass allocation, adventitious rooting, aerenchyma formation and ethylene production. Environ. Exp. Bot. 48, 119–128.
- Chen, H., Qualls, R.G., 2003. Anaerobic metabolism in the roots of seedlings of the invasive exotic *Lepidium latifolium*. Environ. Exp. Bot. 50, 29–40.
- Crawford, R.M.M., 1978. Metabolic adaptation to anoxia. In: Hook, D.D., Crawford, R.M.M. (Eds.), Plant Life in the Anaerobic Environment. Ann Arbor Scientific Publishing, Ann Arbor, MI, pp. 119–136.
- Drew, M.C., 1988. Effects of flooding and oxygen deficiency on plant mineral nutrition. In: Lauchli, A., Tinker, P.B. (Eds.), Advances in Plant Nutrition, vol. III. Praeger Publishing, New York, pp. 115–159.
- Dubois, M., Giles, K.A., Mamilton, J.K., Rebers, P.A., Smith, F., 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28, 350–356.
- Edwards, D.G., Asher, C.J. 1982. Tolerance of crop and pasture species to manganese toxicity. In: Scaife, A., (Ed.), Proceedings of the Ninth Plant Nutrition Colloquium, Warwick, England, Commonwealth Agricultural Bureau, Farnham Royal, Bucks, UK, pp. 145–150.
- Eise, M.A., Hall, K.C., Arnold, G.M., Davies, W.J., Jackson, M.B., 1995. Export of abscisic acid, 1-aminocyclopropane-1-carboxylic acid, phosphate and nitrate from roots to shoots of flooded tomato plants. Plant Physiol. 107, 377–384.
- Fredrickson, L.H., Diebboll, R., Harvey, L., Rilling, R., Laubhan, M.K., 1999. Response of tall whitetop to land management practices in San Luis Valley, CO, National Symposium on Tall Whitetop, Alamosa, CO, pp. 43–45.
- Gambrell, R.P., DeLaune, R.D., Patrick Jr., W.H., 1991. Redox processes in soils following oxygen depletion. In: Jackson, M.B., Davies, D.D., Lambers, H. (Eds.), Plant Life under Oxygen Deprivation: Ecology, Physiology and Biochemistry. SPB Academic, The Hague, pp. 101–117.
- Geiger, D.R., Savonick, S.A., 1975. Effect of temperature, anoxia, and other metabolic inhibitors on translocation. In: Zimmermann, M.A., Milburn, J.A. (Eds.), Transport in Plants: I. Phloem Transport, Encyclopedia of Plant Physiology New Series. Springer-Verlag, New York, pp. 256–286.

- Gravatt, D.A., Kirby, C.J., 1998. Patterns of photosynthesis and starch allocation in seedlings of four bottomland hardwood tree species subjected to flooding. *Tree Physiol.* 18, 411–417.
- Graven, E.H., Attoe, O.J., Smith, D., 1965. Effect of liming and flooding on manganese toxicity in alfalfa. *Soil Sci. Soc. Am. Proc.* 29, 702–706.
- Hendry, G.A.F., Brocklebank, K.J., 1985. Iron-induced oxygen radical metabolism in waterlogged plants. *New Phytol.* 101, 199–206.
- Hook, D.D., Brown, C.C., 1973. Root adaptations and relative flood tolerance of five hardwood species. *Forensic Sci.* 19, 225–229.
- Hunt, R., 1982. *Plant Growth Curves: The Functional Approach to Plant Growth Analysis*. Edward Arnold Ltd., London, pp. 14–33.
- Jones, R., 1972. Comparative studies of plant growth and distribution in relation to waterlogging: V. The uptake of iron and manganese by dune and dune slack plants. *J. Ecol.* 60, 131–139.
- Kozlowski, T.T., 1984. Plant responses to flooding of soil. *Bioscience* 34, 162–167.
- Lindsay, K., 1991. *Plant Tissue Culture Manual*. Kluwer Academic Publishers, p. A1/23.
- Lizaso, J.I., Melendez, L.M., Ramires, R., 2001. Early flooding of two cultivars of tropical maize: II. Nutritional responses. *J. Plant Nutr.* 24, 997–1011.
- Marschner, H., 1995. *Mineral Nutrition of Higher Plants*. Academic Press, pp. 626–641.
- McKevlin, M.R., Hook, D.D., McKee Jr., W.H., Wallace, S.U., Woodruff, J.R., 1987. Phosphorus allocation in flooded loblolly pine seedlings in relation to iron uptake. *Can. J. For. Res.* 17, 1572–1576.
- Munz, P.A., Keck, D.D., 1959. *A California Flora*. University of California Press, Berkeley, CA, 1681 pp.
- Murphy, J., Riley, J.P., 1962. A modified single solution method for the determination of phosphate in natural waters. *Anal. Chim. Acta* 27, 31–36.
- Pallas, J.R., Kays, S.J., 1982. Inhibition of photosynthesis by ethylene—a stomatal effect. *Plant Physiol.* 70, 598–601.
- Pezeshki, S.R., 1995. Plant responses to flooding. In: Wilkinson, R.E. (Ed.), *Plant–Environment Interactions*. Marcel Dekker, New York, pp. 289–321.
- Pezeshki, S.R., 2001. Wetland plant responses to soil flooding. *Environ. Exp. Bot.* 46, 299–312.
- Pezeshki, S.R., DeLaune, A.D., Anderson, P.H., 1999. Effect of flooding on elemental uptake and biomass allocation in seedlings of three bottomland tree species. *J. Plant Nutr.* 22, 1481–1494.
- Pezeshki, S.R., DeLaune, A.D., Klude, H.K., Choi, H.S., 1996a. Photosynthesis and growth responses of cattail (*Typha domingensis*) and sawgrass (*Cladium jamaicense*) to soil redox conditions. *Aquat. Bot.* 54, 25–35.
- Pezeshki, S.R., Pardue, J.H., DeLaune, R.D., 1996b. Leaf gas exchange and growth of flood-tolerant and flood-sensitive tree species under low soil redox conditions. *Tree Physiol.* 16, 453–458.
- Ponnamperuma, F.N., 1972. The chemistry of submerged soils. *Adv. Agron.* 24, 29–96.
- Poorter, H., 1989. Plant growth analysis: towards a synthesis of the classical and the functional approach. *Physiol. Plant* 75, 237–244.
- Saglio, P.H., 1985. Effect of path or sink anoxia on sugar translocation in roots of maize seedlings. *Plant Physiol.* 77, 285–290.
- SAS Institute Inc., 1989. *SAS/STAT User's Guide*, version 6, fourth ed. SAS Institute Inc., Cary, NC.
- Sij, J.W., Swanson, C.A., 1973. Effect of petiole anoxia on phloem transport in squash. *Plant Physiol.* 51, 368–371.
- Taylor, G.E., Gunderson, C.A., 1988. Physiological site of the ethylene effects on carbon dioxide assimilation in *Glycine max* L. Merr. *Plant Physiol.* 86, 85–92.
- Thomson, C.J., Atwell, B.J., Greenway, H., 1989. Response of wheat seedlings to low O<sub>2</sub> concentrations in nutrient solution: II. K<sup>+</sup>/Na<sup>+</sup> selectivity of root tissue of different age. *J. Exp. Bot.* 40, 993–999.
- Topa, M.A., Cheeseman, J.M., 1992. Carbon and phosphorus partitioning in *Pinus serotina* seedlings growing under hypoxic and low-phosphorus conditions. *Tree Physiol.* 10, 195–207.
- Topa, M.A., Cheeseman, J.M., 1993. <sup>32</sup>P uptake and transport to shoots in *Pinus serotina* seedlings under aerobic and hypoxic growth conditions. *Physiol. Plant* 87, 125–133.
- Trought, M.C.T., Drew, M.C., 1980. The development of waterlogging damage in wheat seedling (*Triticum aestivum* L.): II. Accumulation and redistribution of nutrients by the shoot. *Plant Soil* 56, 187–199.
- Vu, J.C.V., Yelenosky, G., 1991. Photosynthetic responses of citrus trees to soil flooding. *Physiol. Plant* 81, 7–14.
- Wample, R.L., Davies, R.W., 1983. Effect of flooding on starch accumulation in chloroplasts of sunflower (*Helianthus annuus* L.) *Plant Physiol.* 73, 195–198.

- Wample, R.L., Thornton, R.K., 1984. Differences in the responses of sunflower (*Helianthus annuus*) subjected to flooding and drought stress. *Physiol. Plant* 61, 611–616.
- Webb, T., Armstrong, W., 1983. The effects of anoxia and carbohydrates on the growth and viability of rice, pea and pumpkin root. *J. Exp. Bot.* 34, 579–603.
- Yamauchi, M., 1989. Rice bronzing in Nigeria caused by nutrient imbalances and its control by potassium sulfate application. *Plant Soil* 117, 275–286.
- Young, J.A., Turner, C.E., James, L.F., 1995. Perennial pepperweed. *Rangelands* 17, 121–123.